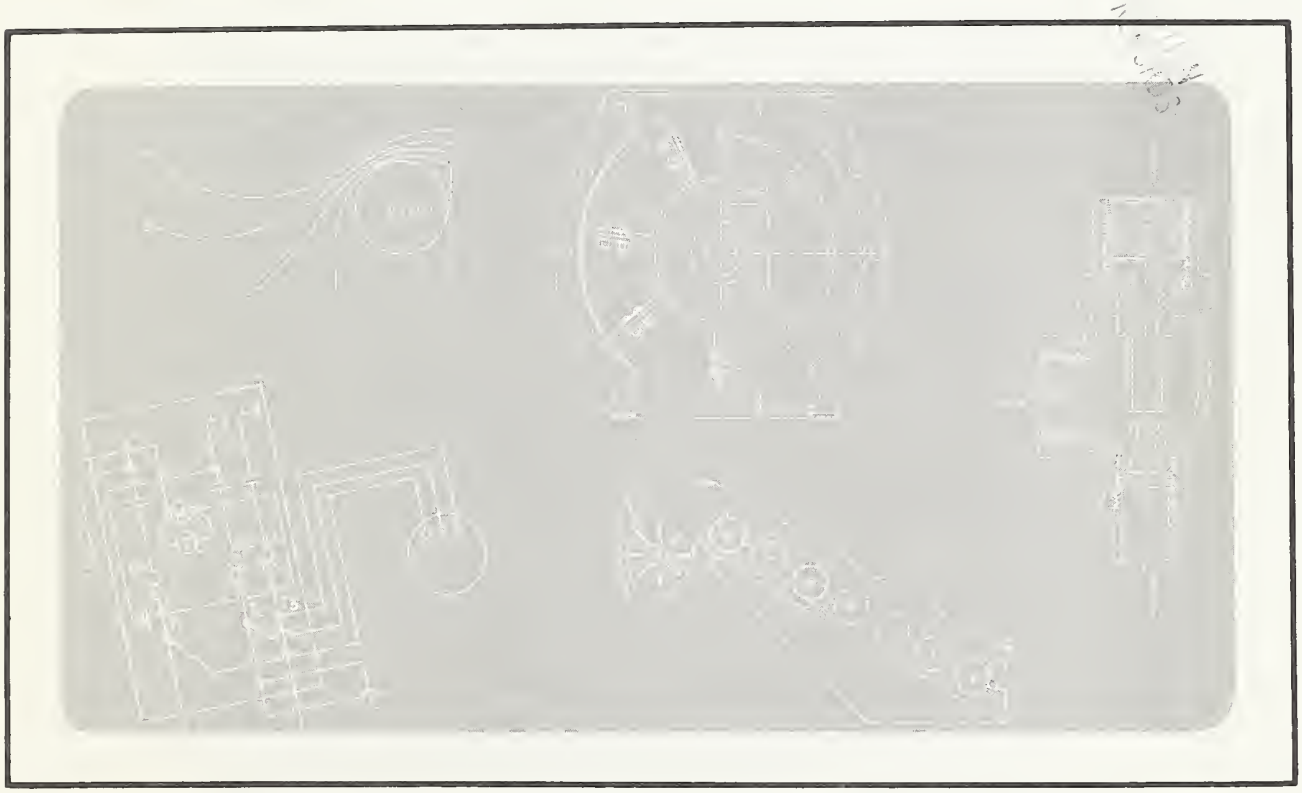
Production of the Tachinid *Lixophaga diatraeae* on Its Natural Host, the Sugarcane Borer, and on an Unnatural Host, the Greater Wax Moth



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Production of the Tachinid *Lixophaga diatraeae* on Its Natural Host, the Sugarcane Borer, and on an Unnatural Host, the Greater Wax Moth

By E. G. King,[1] G. G. Hartley,[2] D. F. Martin,[1] J. W. Smith,[1] T. E. Summers,[3] and R. D. Jackson[4]

ABSTRACT

Larvae of the sugarcane borer, *Diatraea saccharalis* (F.), were reared on a modified soybean flour-wheat germ diet in 22.5-ml cups at a density of two to three larvae per cup. Production was programed for holding eggs and larvae at 28° C and 80% RH in complete darkness and pupae and moths at 24° to 26° C and 80% RH on a 14- to 10-hour light-to-darkness schedule. Larvae of the greater wax moth, *Galleria mellonella* (L.), were reared in 3.8-l jars containing a modified cereal diet with wheat germ. Eggs and larvae were held at 30° C and 50% RH in complete darkness. New techniques were developed for mass harvesting of larvae from jars for parasitization by *Lixophaga diatraeae* (Townsend) and for emerging, mating, and ovipositing (including scale collecting). Adult parasites (flies) were held in gauze-covered cages at 26° C and 80% RH on a 14- to 10-hour light-to-darkness schedule, while parasitized host larvae were held at 26° to 28° C. The flies were rapidly aspirated from the cages, and the parasitic maggots were extracted from the flies with a blender. These maggots were suspended in a 0.15% agar-water solution and injected into cups containing sugarcane borer larvae or were poured into large fiberglass pans along with greater wax moth larvae. Mechanized methods were developed for harvesting the parasite puparia from the pans but not from the cups. A 0.7% formalin wash of maggots during blender extraction effectively eliminated the bacterium *Serratia marcescens*, which caused parasitized host larvae to die from septicemia. Type and intensity of light typically did not affect fly mating, but they did not mate in complete darkness. Also, fly densities of 100 to 1,200 per cage did not affect mating. Preliminary data indicated that parasitized host larvae could be stored at 13° C for 13 days and fly puparia at 15.6° C for 14 days. Thus, total time in storage could be 27 days. About 4.4 million parasites were produced during the sugarcane-growing season (March to mid-October, 1973–76). Production of *L. diatraeae* on greater wax moth larvae reduced costs by 81% when compared with costs of their

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production on sugarcane borer larvae. Facility requirements included several rooms for isolating life stages of the three insects, but clean-room conditions were required only where the soybean flour-wheat germ diet was mixed, dispensed, and infested. Development of this technology enabled conducting of tests to determine the feasibility of controlling sugarcane borer populations on sugarcane in Louisiana and Florida with periodic releases of *L. diatraeae*. Index terms: biological control, *Diatraea saccharalis* (F.), *Galleria mellonella* (L.), insect parasitization, insect rearing, insect-rearing facilities, *Lixophaga diatraeae* (Townsend).

INTRODUCTION

Knipling (1972) proposed that properly timed releases of the parasite *Lixophaga diatraeae* (Townsend) might control the sugarcane borer (SCB), *Diatraea saccharalis* (F.), a major pest of sugarcane in Louisiana, Texas, Florida, and Central and South America. *Lixophaga diatraeae*, a larviparous tachinid, is native to several Caribbean Sea islands but has been colonized in other areas, including the States of Louisiana and Florida (Bennett 1969). Charpentier et al. (1971), however, reported that *L. diatraeae* had not become permanently established in Florida despite numerous attempts to establish the parasite there.

During 1973–74 we developed a rearing program for *L. diatraeae*, using only SCB larvae as the host, to test Knipling's hypothesis in the field (King et al. 1975c, McPherson and Hensley 1976, and Summers et al. 1976). However, in 1975 we used larvae of the greater wax moth (GWM), *Galleria mellonella* (L.), though not a natural host (Montes 1970, Summers et al. 1971, and Guerra 1974), as hosts for *L. diatraeae* because they were easier and less expensive to mass-produce. Thus, we had to maintain three reproductive (brood) colonies (fig. 1). We maintained the fly (parasite) reproductive colony on SCB larvae, its natural host, but mass-produced the fly on GWM larvae. We used maggots from flies reared on SCB larvae to parasitize SCB and GWM larvae. Maggots from flies reared on GWM larvae could also be used to parasitize additional GWM larvae for mass production.

We report herein on procedures and devices developed for producing *L. diatraeae* on SCB and GWM larvae. Also, methods for host rearing are presented, including previously unpublished data on disinfecting *L. diatraeae* maggots and mating and storage of *L. diatraeae*. This program was briefly summarized in Morrison and King (1977), and some of the devices developed for mechanized rearing were reported by Gantt et al. (1976) and Hartley et al. (1977). The program has been discontinued, and the facilities are being converted for use in another insect-rearing program.

HOST REARING

Sugarcane Borer

There are numerous reports on artificial diets with various procedures for rearing *D. saccharalis* (Singh 1977). Our rearing procedures were derived

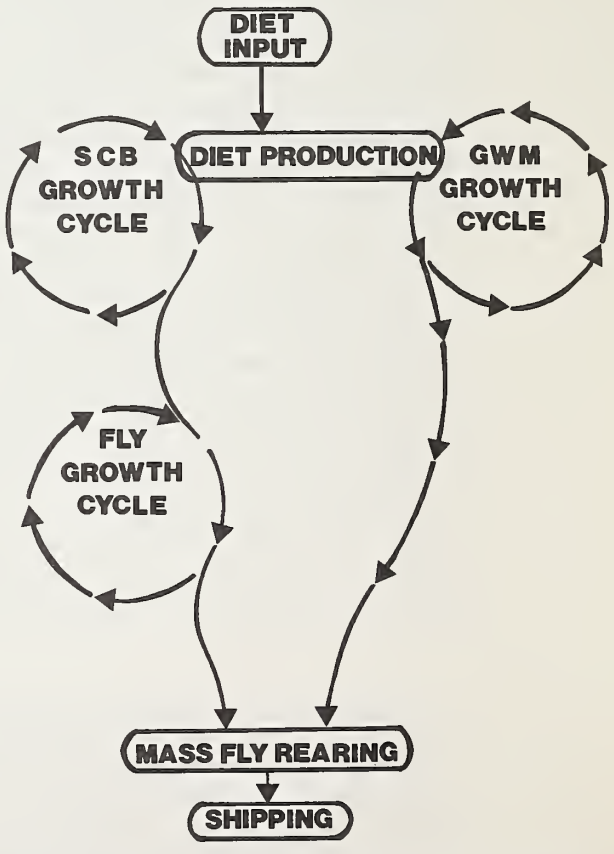

Figure 1.—Flow chart showing system for producing *L. diatraeae* on sugarcane borer and greater wax moth larvae.

from those reported by Hensley and Hammond (1968) and Brewer (1976). The diet reported by Brewer (1976) was further simplified (table 1).

A flow chart of the SCB production system is given in figure 2. The SCB larvae were reared in 22.5-ml plastic cups (Thunderbird Corp., El Paso, Tex.) on 5 to 7.5 ml of the soybean flour-wheat germ diet. Wax-impregnated cardboard lids (Standard Cap and Seal, Chamblee, Ga.) were used to cover the cups because large SCB larvae frequently chewed through the plastic lids. Larvae used solely for moth production (colony maintenance and increase) were reared at a density of two per cup, because any increase in the number of larvae per cup resulted in damage to pupae from the chewing of unpupated larvae, thus offsetting any increase of pupae (King et al. 1975c). Conversely, larvae used solely in parasite production were reared at a density of three per cup, since they were parasitized in the early fifth stage and consequently became less active and less likely to injure other host larvae. The larvae required 20 days at 28° C to develop to the pupal stage, but for parasite rearing they were exposed when 14 days old, or early in the fifth stage (Miles and King 1975).

The pupae were removed from the cups by hand, disinfected with 4% formalin for 2 minutes, and then rinsed thoroughly in distilled water. The pupae were sexed, and males and females were placed in separate 4-l cardboard containers (200 to 300 pupae per container) containing a small amount of moistened vermiculite. The top of each container was replaced with nylon organdy. (Sexing was not 100% accurate since it was based on size, the female pupae being larger than the male pupae.)

As the moths emerged they were transferred to a similar container (40 pairs per container) lined with wax paper, which served as an oviposition surface. A water-soaked piece of cotton placed on the organdy served as a water source; a carbohydrate source was not provided. Beginning the second day after emergence (female moths mate on the first night after emergence and begin ovipositing on the second night) oviposition liners were replaced daily for four consecutive nights. The moths were then discarded because of reduced egg production and moth mortality (King et al. 1975b).

The wax-paper lining (inserts) with eggs were washed in 3.3% formalin for 10 minutes, rinsed thoroughly in distilled water for 15 minutes, and air-dried beneath a laminar-flow hood (clean air) until almost dry. The egg-covered wax-paper linings were then placed into sterile 2-l Erlenmeyer flasks containing moist cotton. The flasks were plugged with sterile cotton, wrapped in aluminum foil, and held until eclosion. The newly hatched larvae, being positively phototactic, moved to the top of the flask, where they were removed with a

Table 1.—Composition of soybean flour-wheat germ diet for rearing sugarcane borer larvae[1]

Ingredient	Amount	Source
Wheat germ	32 g	Nutritional Biochemicals Corp., Cleveland, Ohio.
Soybean flour (Nutrisoy)	37 g	Archer Daniels Midland Co., Decatur, Ill.
Sucrose	32 g	
Wesson salt	9 g	Nutritional Biochemicals Corp., Cleveland, Ohio.
Chlortetracycline (Aureomycin)[2]	1.05 g	American Cyanamid Co., Princeton, N.J.
Sorbic acid	1 g	Sigma Chemical Co., St. Louis, Mo.
Alphacel	4.7 g	Nutritional Biochemicals Corp., Cleveland, Ohio.
Methyl-*p*-hydroxybenzoate	1.6 g	Sigma Chemical Co., St. Louis, Mo.
Vitamin Premix (custom)	10.6 g	Roche Chemical Division, Hoffman-La Roche Inc., Nutley, N.J.
Water in blender	849 ml	
Agar	22 g	Perney Inc., Ridgewood, N.J.

[1]Mixing instructions for 1 l of diet. Premix all dry material except agar and place in blender. Dissolve agar in 849 ml of hot water, heat to boiling, and add to blender with dry material. Blend solution for 4 minutes and pour into cups.

[2]Used as a bactericide and may be replaced by other possibly more effective antibiotics against specific bacteria after suitable bioassays.

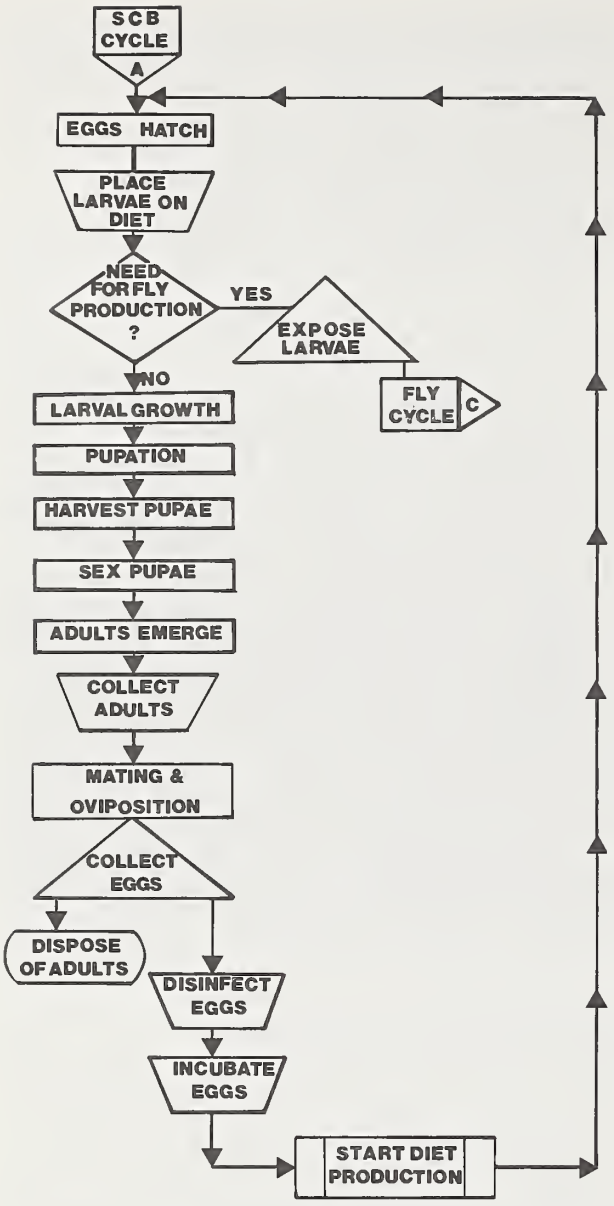

FIGURE 2.—Flow chart showing the sugarcane borer production system.

fine brush and transferred to the cups containing diet.

For rearing purposes, eggs and larvae were held at 28° C and 80% RH in complete darkness. Harvested pupae and moths were held at 24° to 26° C and 80% RH on a 14- to 10-hour light-to-darkness (14 : 10 L : D) cycle. Under these conditions the eggs required 5 to 6 days, the larvae 19 to 21 days, and the pupae about 8 days to complete development. The eggs were stored at 15.6° C to prevent hatching over weekends. We earlier reported on the effects of temperatures (15.6° to 32° C) on the life stages (King et al. 1975b).

Greater Wax Moth

Our methods for rearing the GWM were similar to those reported by Dutky et al. (1962), except the diet was modified and the procedures were greatly expanded. Diets were reviewed for rearing the GWM (Marston and Campbell 1973). Marston et al. (1975) reported on an inexpensive diet containing a whey-grown yeast product (Wheast). This diet was satisfactory for rearing GWM larvae to produce *L. diatraeae*, but Wheast is no longer available and a comparable product is too expensive. The diet reported by Dutky et al. (1962) was unsatisfactory (unpublished data) but was fortified with Gerber high-protein cereal and wheat germ. (table 2). Using this diet resulted in a parasite comparable to those produced on SCB larvae; however, before this change in diet fly emergence from puparia and adult female parasite longevity were less when parasites were reared on GWM larvae than when reared on SCB larvae. Etienne (1973) reported that *L. diatraeae* could not be continuously reared on GWM larvae that were fed beeswax and pollen but could be when the host diet was supplemented with vitamin E or wheat germ (Etienne 1974). The GWM larvae can serve as an unnatural host for a

Table 2.—Composition of cereal diet for rearing greater wax moth larvae[1]

Ingredient	Amount	Source
Gerber mixed cereal	187 g	Gerber Products Co., Manufacturers, Fremont, Mich.
Gerber high-protein cereal ..	187 g	Do.
Wheat germ	120 g	Nutritional Biochemicals Corp., Cleveland, Ohio.
Sucrose	148 g	
Glycerol	219 g	Dow Chemical Co., Midland, Mich.
Vitamin mix (Poly-Vi-Sol) ..	1.2 ml	Mead Johnson & Co., Evansville, Ind.
Water	138 g	

[1]Mixing instructions for 1 kg of diet. Dissolve sugar (sucrose) in heated water. Add glycerol. Cool mixture and add vitamin mix. Add liquid to dry ingredients and mix about 1 minute.

number of tachinid species (Campadelli 1975), including *L. diatraeae* (Bennett 1969, Montes 1970, Summers et al. 1971, Guerra 1974, and Morrison and King 1977).

The GWM production system is given in figure 3. The larvae were reared in 3.8-l glass or plastic jars in 750 g of diet. Forty-eight milligrams of eggs (32 mg = 1,000 eggs) were placed on the diet surface of each jar. Screen covered with filter paper was used for each jar top and was attached by the screw cap with the center removed. The filter prevented escape of the newly hatched larvae and entrance of some contaminants, while the screen prevented escape of large larvae; both allowed ventilation. The jars of eggs and developing larvae were held at 30° C and 50% RH in complete darkness (fig. 4). After about 37 days (duration of the egg stage was about 6 days), the larvae moved to the top of the jars to spin cocoons. These larvae were harvested on alternate days to obtain insects of about the same physiological age for the reproductive colony. Larvae from jars used for parasite production were harvested once. Larvae used solely for the reproductive colony were placed in 1-l glass jars (300 larvae per jar). The jar lids were replaced with household window screen to provide ventilation but prevent larval escape. (Good ventilation is required to allow release of excess moisture accumulating from the emptying of larval gut tracts before cocoon formation.) After the larvae pupated, the jar lids were removed, and each jar was placed in a

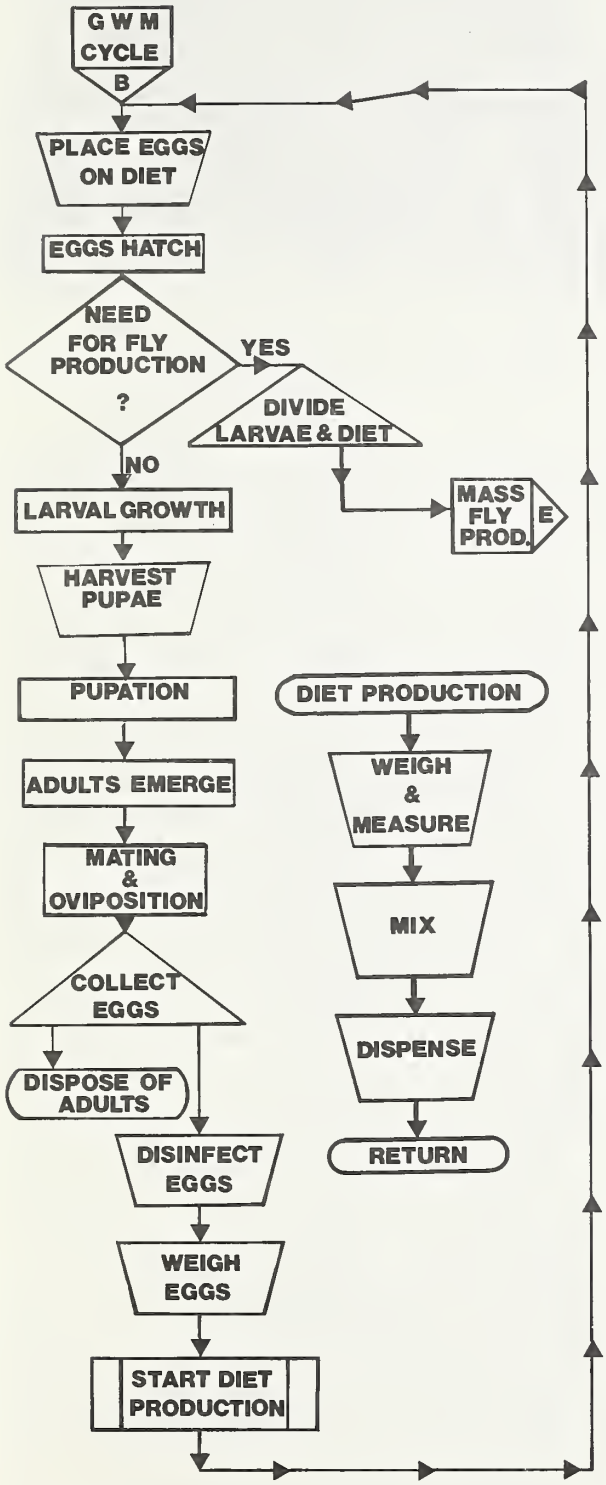

FIGURE 3.—Flow chart showing the greater wax moth production system.

FIGURE 4.—Jars in the larval rearing room containing eggs and developing larvae of the greater wax moth.

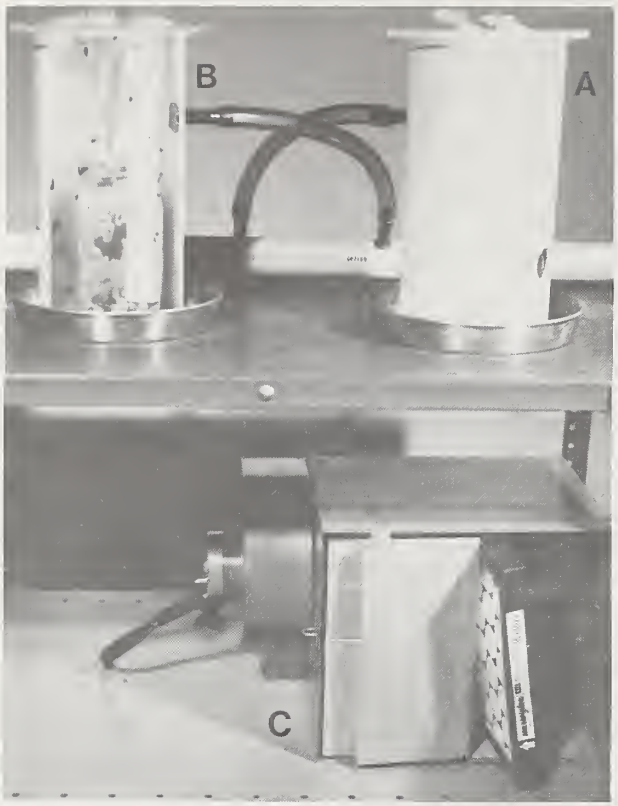

FIGURE 5.—Oviposition chambers and moth-scale collector. A, Chamber. B, Cutaway view of chamber showing wax paper sheets for oviposition and glass jar containing pupal cases. C, Blower with filter for collecting scales.

chamber that served for emerging, mating, and ovipositing (fig. 5, A and B).

Hartley et al. (1977) described the oviposition chamber and method for scale collecting. Large quantities of loose scales occur in GWM production, and these are a health hazard (Ridgway and Whittam 1970). A hole near the top of each chamber allows air intake, and another hole with a hose attached at the bottom provides for an air outlet. Airflow through the chamber is created by a blower attached to a rigid manifold that is connected to each chamber outlet by a flexible hose. The blower pulls air through the chambers and collects the airborne moth scales on a filter (C).

Each female moth deposited about 700 eggs over a period of approximately 7 days in accordion-folded strips of wax paper that were stapled at each end. After 7 days the moths were discarded. The wax-paper sheets were replaced three times

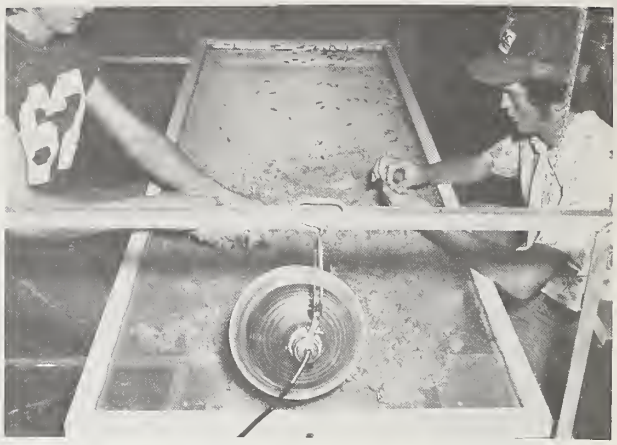

FIGURE 6.—Greater wax moth larvae harvester and 150-W floodlight. Operators are shredding diet to release larvae.

weekly, and the eggs were collected by scraping them from the sheet. These eggs were disinfected with 3.3% formalin for 10 minutes (6.4% formalin can also be used if a greater concentration is desired), thoroughly rinsed in distilled water, air-dried, and partitioned into vials by weight for later transfer to the jars containing diet. Egg hatch was greater than 90%.

Larvae used for *L. diatraeae* production were harvested from the jars just before cocoon formation. Each jar contained about 1,000 healthy larvae, each of which weighed between 200 and 300 mg, plus larval silk, frass, diet, and dead or injured larvae. The jar contents were emptied onto one end of a screen framed by wooden strips (fig. 6). The diet was shredded by hand, and a floodlight directed on the material caused the negatively phototactic larvae to move to the unlighted end. A wire strung around the inside of the frame was heated to 90.6° C by a variable transformer supplying 68 V a.c. to prevent larval escape. Finney et al. (1947) and Hollingsworth et al. (1961) also used heat as a barrier to form an enclosure pen for insects. The enclosed larvae at the unlighted end were easily aspirated into a jar attached to a modified vacuum sweeper and transferred to the parasitization room. Larval counts were based on volumetric measurement where 500 ml equaled about 2,000 full-grown larvae. We were able to store these larvae at low temperatures for short periods of time, as reported by Dutky et al. (1962).

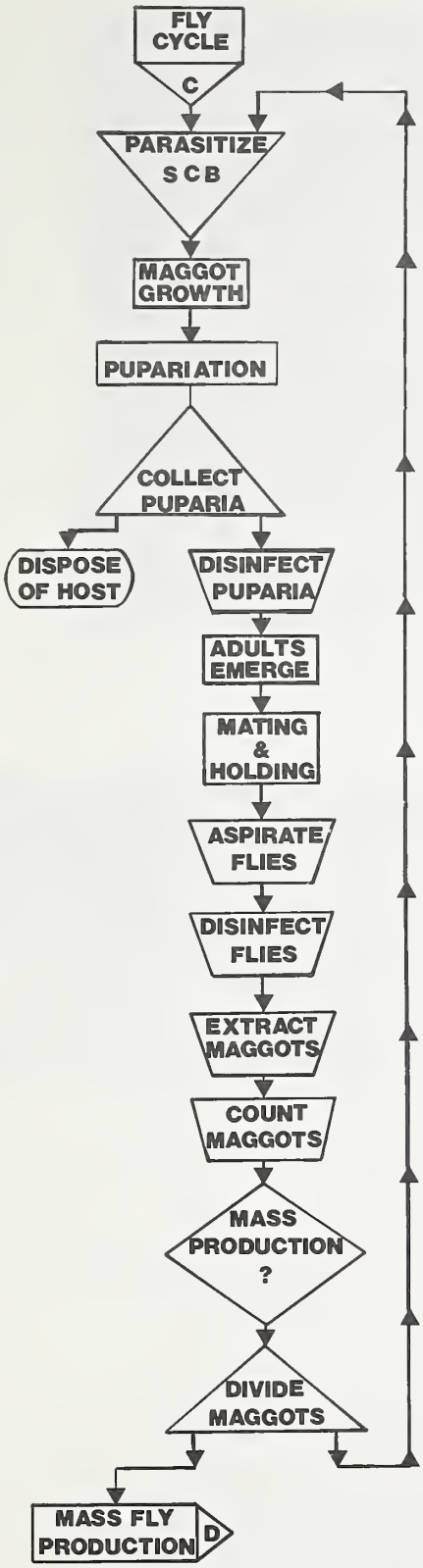

FIGURE 7.—Flow chart showing system for producing *L. diatraeae* on sugarcane borer larvae.

PARASITE REARING

Laboratory rearing procedures for *L. diatraeae* were summarized by Bennett (1969). The flies were held in cages for emergence and mating, and until maggot extraction. (*L. diatraeae* is larviparous, and maggots are physically removed from the fly.) Food and water were supplied to the flies daily. The uteri were dissected from the flies and placed in a water droplet; as the maggots eclosed from eggshells, one to three maggots were placed on each host larva with a fine brush. We modified these procedures, greatly increasing production potential on SCB larvae (King et al. 1975c). We later developed a system for producing the parasite on GWM larvae.

The system for producing *L. diatraeae* on SCB larvae is given in figure 7. Adult flies emerged and were held in 25.4- by 22.9- by 15.2-cm aluminum-frame cages covered with Tubegauz (Scholl Manufacturing, Inc., Chicago, Ill.). Each cage contained a watering device consisting of a 400-ml glass of water inverted over a Plexiglas plate covered with a screen (fig. 8). The water was released slowly onto the screen, where it collected in droplets in the screen holes and thereby provided a water source to the flies. Additional water was sprinkled over

FIGURE 8.—Cage for holding *L. diatraeae* flies, including watering device and bottle with puparia for fly emergence.

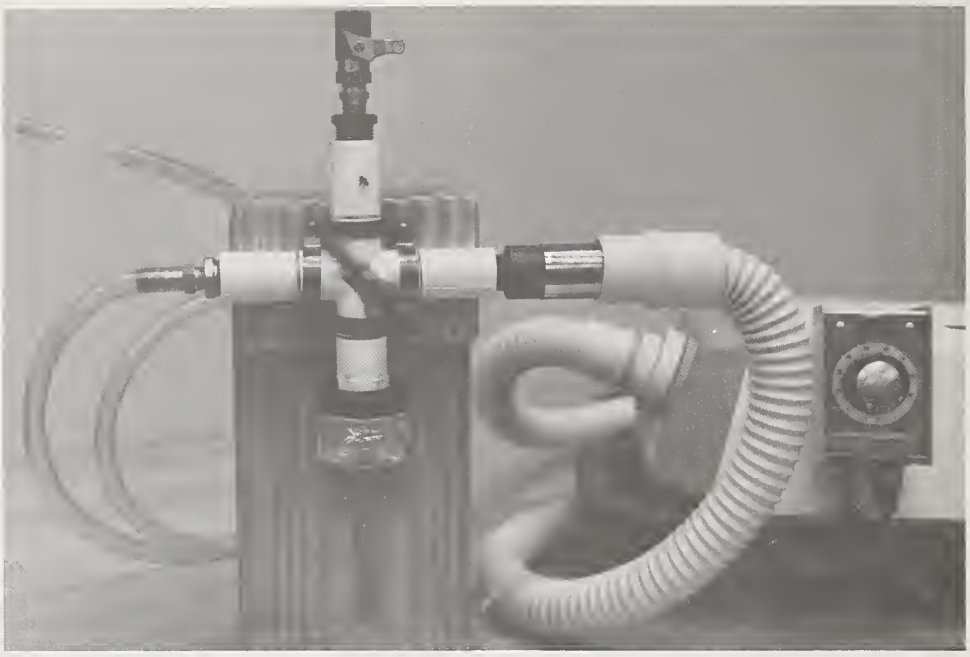

FIGURE 9.—Aspiration device for transferring *L. diatraeae* adults from holding cage to jar containing 1% NaOCl.

the cage surface three times daily during weekdays, and raw sugar (food source) was sprinkled on the upper cage surface as needed. When sugar was withheld, mortality approached 100% after 7 days, but when sugar was provided, more than 90% of the flies usually survived for 11 days. The flies were held at 26° C and 80% RH on a 14 : 10 L : D cycle.

The adult flies were held until they were 12 to 14 days old or until the maximum number of maggots could be extracted (King and Martin 1975), which was the latter part of the peak larviposition period (Roth and King 1978). The flies were aspirated into a collection jar (fig. 9) containing 1% sodium hypochlorite (NaOCl) plus 0.16% Photo Flo (Eastman Kodak Co., Rochester, N.Y.) for 3 minutes. (Photo Flo served as a wetting agent.) The flies were then rinsed with distilled water. (Maggots in the uterus are not damaged during this treatment, and the external surface of the flies is sterilized). After the NaOCl was rinsed from the flies, 200 to 800 flies were placed with 50 ml of 0.7% formalin solution into a stainless-steel semimicro container that could be used with a commercial Waring Blendor, which was attached to a rheostat autotransformer that produced 8,500 r/min at 45 V a.c. The flies were blended three times for 3 seconds each time, which separated the fly abdomen from the thorax and ruptured the uterus, thereby releasing the maggots into the formalin solution.

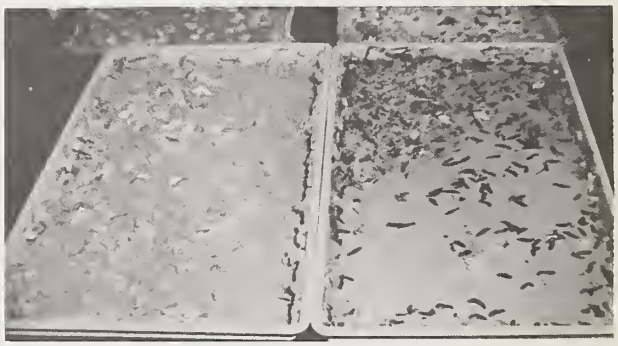

FIGURE 10.—Greater wax moth larvae in trays exposed to contaminated *L. diatraeae* maggots or exposed to maggots washed in 0.7% formalin solution. Most of host larvae in tray on right died from septicemia, caused by *S. marcescens*, because maggots were not extracted in formalin solution. Larvae in tray on left cocooned normally and maggots emerged to form puparia, as shown in figure 14.

We previously reported on a bacterium, *Serratia marcescens* (Bizzio), that often contaminated the maggots and caused septicemia in parasitized SCB larvae (King et al. 1975a). This was even more severe among parasitized GWM larvae. The formalin and washing process effectively eliminated the bacterium (fig. 10). Based on trial and error we found that rates of formalin greater than 0.7% or wash periods exceeding 5 minutes resulted in

Table 3.—Greater wax moth larvae parasitized by *L. diatraeae* maggots and maggot survival after washing in three concentrations of formalin for 5 or 10 minutes[1]

Measurement	Check	Concentration of formalin (pct)					
		5-Minute wash period			10-Minute wash period		
		0.4	0.8	1.6	0.4	0.8	1.6
Larvae parasitized ... % ···	72	79	81	52	85	53	0
Maggot survival ..... % ···	67	71	77	39	80	31	0

[1]25 greater wax moth larvae were placed in each of 3 petri dishes (1 petri dish = 1 replication) containing 50 maggots that had been extracted in sterile distilled water (check) or 0.4%, 0.8%, or 1.6% formalin and washed for 5 or 10 minutes, followed by a thorough rinse and suspension in 0.15% agar solution. Values are averages of 3 replications. Maggot survival was development to pupal stage.

maggot mortality, and that rates of formalin less than 0.6% were ineffective against the bacterium. Our experimental data tended to substantiate these observations (table 3). Ethanol, NaOCl, and nalidixic acid were also tested as maggot washes at various concentrations, but they were ineffective or they killed the maggots (unpublished data).

The maggots were washed for 5 minutes in the formalin, and then the solution was poured through a 24-mesh stainless-steel screen into a beaker. The screen retained the larger fly particles but allowed the maggots and supernatant to pass through. The formalin solution was decanted, and the maggots were thoroughly rinsed with sterile distilled water (maggots sink). The maggots were subsequently suspended in 0.15% agar solution, which was placed in a petri dish that was positioned over a grid pattern. There the maggots that had exited the egg chorion were counted in 11 randomly selected squares (0.4 cm^2 per square). The average maggot count was calculated per sample square and multiplied by 171 (number of 0.4 cm^2 per dish) to obtain the total number of maggots per dish. Additional agar solution was added as needed to obtain the desired maggot density in a given solution.

When *L. diatraeae* was reared on SCB larvae, the agar-water solution containing the maggots was metered in 0.3-ml droplets into 22.5-ml cups containing early fifth-stage host larvae at a density of three to four maggots per host larva (fig. 11). After a series of laboratory tests (Miles and King 1975, King et al. 1976), this host stage was selected as best for rearing *L. diatraeae*. The dispenser consisted of a positive-displacement piston pump that was activated by a solenoid switch attached to a discharge needle that energized the motor automatically by penetrating the cup lid (Gantt et al. 1976). For smaller scale rearing, a 2.5-ml repeating dispenser (Hamilton model PB 600–1) was used (King et al. 1975c).

The maggots sought out the host larvae feeding on the artificial diet in the cup. They typically entered the host larvae through an intersegmental membrane and within about 48 hours attached to the lateral tracheal trunks, where they completed development while feeding on hemolymph, fat bodies, and muscle tissue (King et al. 1976).

The system for producing *L. diatraeae* on GWM larvae is given in figure 12. The larvae were parasitized just before cocoon formation; thus, no further food was required after harvesting from the diet. They were placed in 30.5- by 61.0- by 5.0-cm fiberglass trays (fig. 13) containing 4,000 to 6,000

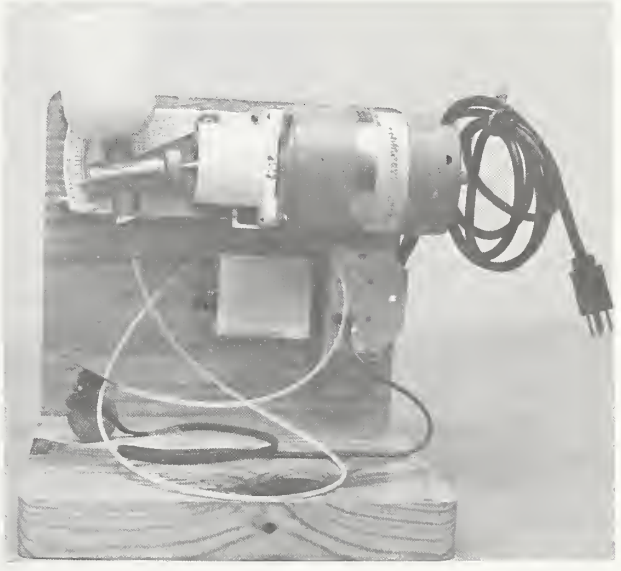

FIGURE 11.—Machine for dispensing *L. diatraeae* maggots suspended in 0.15% agar-water solution into 22.5-ml cups containing sugarcane borer larvae.

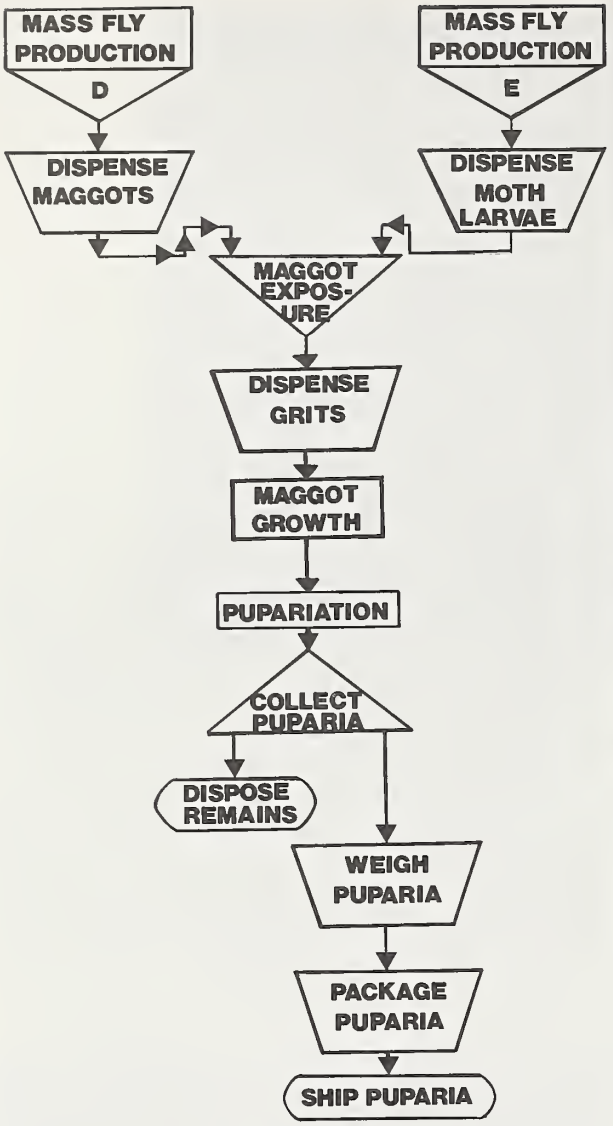

FIGURE 12.—Flow chart showing system for producing *L. diatraeae* on greater wax moth larvae.

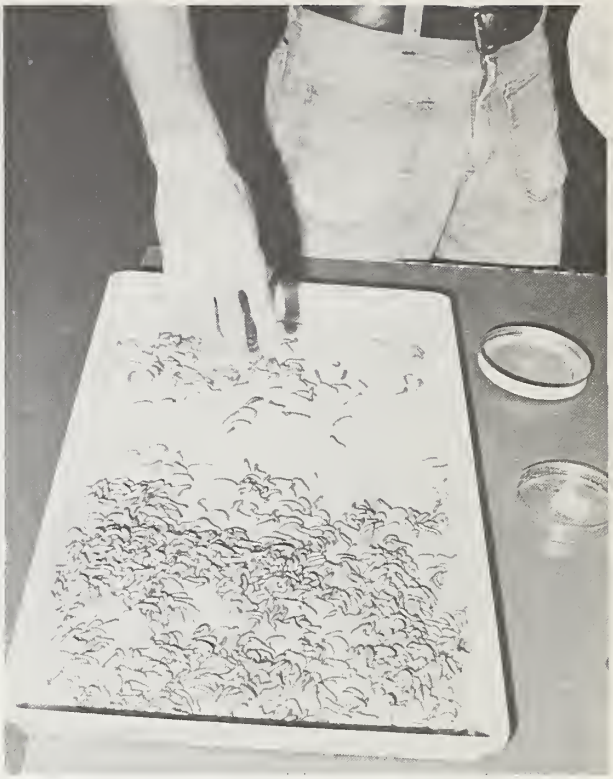

FIGURE 13.—*L. diatraeae* maggots suspended in 0.15% agar-water solution in tray into which greater wax moth larvae were placed.

parasite maggots that had been applied by "pouring" or spraying with an airbrush. Two thousand host larvae were placed in each tray, in which about 5 ml of the agar-water solution containing maggots had been dispensed evenly over the tray bottom. A screen was placed over each tray, and a similar perforated tray was inverted over the tray containing insects. Then each two-tray unit was strapped together. The screen was required for retaining the larvae in each tray, and the perforated tray provided a base for strapping yet still allowed ventilation. After about 1 hour the maggots had entered the host larvae, and 30 ml of corncob grits were added to the trays to absorb the remaining agar solution.

Parasitized larvae were held at 26° to 28° C. The maggots completed development in 6 to 9 days in both the SCB and GWM, and parasite puparia were harvested on the 11th day after parasitization. Puparia were removed with forceps from cups containing SCB larvae. GWM larvae, when parasitized by *L. diatraeae* maggots, usually formed a cocoon but did not pupate. However, about one-half of the maggots emerging from the host larvae remained in the cocoon, thereby necessitating a rapid method for removing the puparia (fig. 14). Only GWM larvae were used in mass production because of reduced expense and ease of production when using these larvae.

Puparia that formed outside of GWM cocoons were harvested by simply brushing them from the trays. Those retained in the host cocoon were harvested by dissolving the cocoon in 1% NaOCl solution at a temperature of 24° C for about 6 minutes (Hartley et al. 1977). Freed puparia floated and thus could be skimmed from the surface, after which they were rinsed thoroughly in 24° C

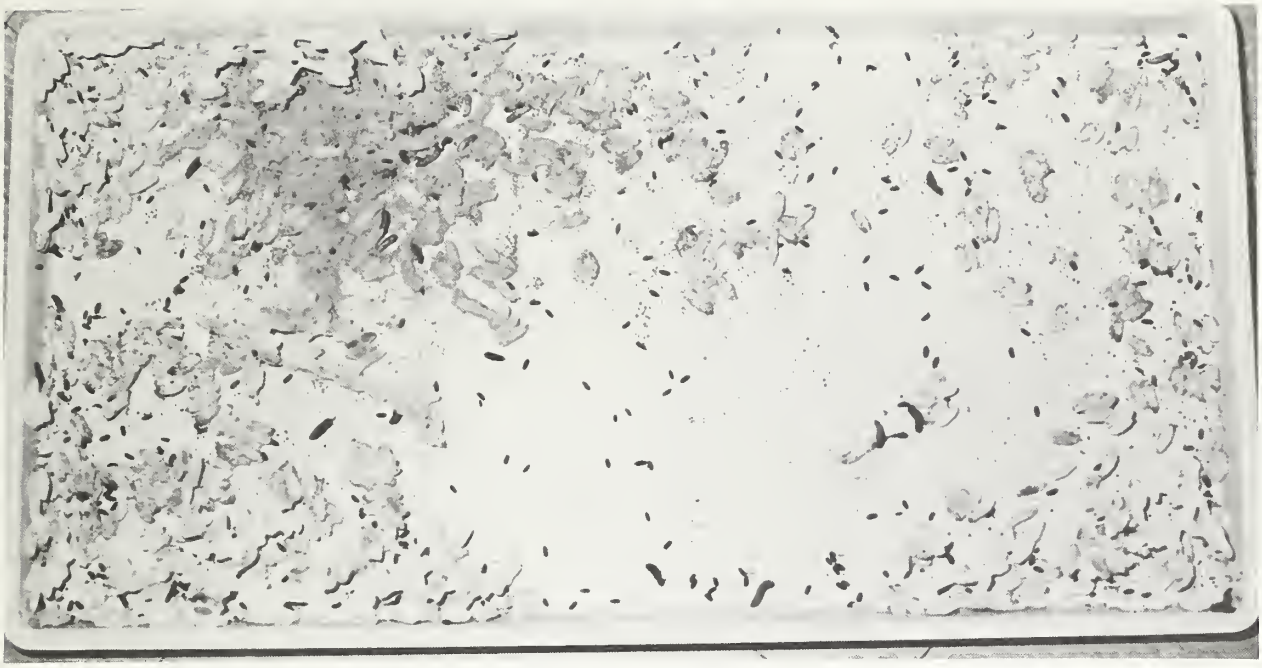

FIGURE 14.—Cocooned greater wax moth pupae in tray with *L. diatraeae* puparia (black seedlike objects).

Table 4.—Percentages of mating for *L. diatraeae* flies reared on two different hosts exposed to various light intensities and light sources[1]

Light source	Light intensity[2](fc)		Average percentage of mating when reared on[3]—	
	Mean	Range	Sugarcane borer	Greater wax moth
GE Chroma 50 + incandescent ...	198	130–275	95	88
GE Chroma 50 + incandescent ...	80	60–100	85	83
Incandescent	13	10–15	90	81
GE cool white	229	115–290	83	79
GE Chroma 50	42	25–55	86	85
GE Chroma 50	174	120–190	72	87
Incandescent	28	20–40	81	89
GE Chroma 50	78	60–100	73	84
GE Chroma 50	118	70–160	89	85
No light	0	.....	5	6

[1]Flies were held at 26° C and 80% RH with 14 hours of light and 10 hours of dark (where light was provided) in tubular gauze covered cages (100 flies per cage). Four cages were held in each light regime. Female flies from each cage were dissected 5 days after emergence from puparia, and their uteri were inspected for eggs. However, mating was confirmed by microscopic examination of the spermatheca for sperm presence, since up to 50% of the virgin females may have contained some eggs in their uterus.

[2]Intensity recorded from light striking tops of cages. Light intensity varied with cage position within regimes, so the average was calculated.

[3]Differences in mating between flies reared on sugarcane borer larvae and those reared on greater wax moth larvae, for all light sources and intensities, are not significant at the 95% level of probability as determined by Student's *t*-test.

Table 5.—Effects of storage of *L. diatraeae* puparia at low temperatures for various time intervals on certain biological parameters[1]

Storage time (days)	Percentage of adult emergence at temperature of—			Percentage of mating at temperature of—			Percentage of female survival at temperature of—			Average No. eggs per female at temperature of—		
	4.4° C	10° C	15.6° C	4.4° C	10° C	15.6° C	4.4° C	10° C	15.6° C	4.4° C	10° C	15.6° C
7	86	93	94	85	93	95	63	90	90	94	105	95
14	62	85	94	79	71	90	58	83	76	([2])	67	91
21	46	74	87	35	3	73	60	50	64	....	([2])	83
28	37	42	83	11	0	([3])	55	27	....	....	....	([2])
35	[4]12	[4]19	([3])	0	0	....	([5])	([5])	....	....	....	....

[1]Each storage period was replicated 3 times with 100 puparia per replicate; a check consisted of puparia and flies held continuously at 26° C. After designated storage periods the puparia were transferred to holding cages (1 replicate per cage) at 26° C, and percentage of emergence, percentage of mating (ten 12-day-old females were dissected from each replicate), percentage of female (12-day-old) survival, and mean number of eggs per 12-day-old female (total eggs counted in female uteri) were determined. Check results were as follows: percentage of adult emergence = 94; percentage of mating = 88; percentage of female survival (12-day-old females) = 84; and mean number eggs per 12-day-old female = 102.

[2]Test discontinued because less than ten 12-day-old females were alive in each replicate.

[3]Test discontinued because flies began emerging after 21 days.

[4]Test discontinued because emergence reduced to zero in some replicates.

[5]Test discontinued because fly survival reduced to zero in some replicates after 12 days.

tapwater. The puparia were air-dried and cleaned of extraneous material, and the total number was determined by weighing (based on known sample counts). Puparia shipped to field stations for fly release were packaged between layers of cotton in cartons and placed in Styrofoam boxes that contained icepacks so that temperatures of 18° to 24° C could be maintained for about 1 day during transit. Puparia retained for the reproductive colony, primarily those produced on SCB larvae, were placed in narrow-necked 240-ml plastic bottles (200 to 800 puparia per bottle) containing a cardboard strip. One bottle was placed in each fly cage. Emerging flies were guided from the jars by the cardboard strip, which also served as a substrate to which the flies could attach and expand their wings (fig. 8).

Mating (table 4) was typically high (70% to 95%) regardless of laboratory light source (fluorescent or incandescent or a combination), or intensity (13 to 198 fc). Also, when flies were held at densities of 100, 200, 400, 800, or 1,200 per cage there was no difference in percentage of mating among densities. In neither study was there a difference in percentage of mating between parasites reared on SCB or GWM larvae.

We found that storage of *L. diatraeae* puparia at 15.6° C for 14 days did not affect adult emergence, female survival, or egg production (table 5). However, storage for 21 days caused some reduction in each category. The flies began emerging after 21 days at 15.6° C. The flies could be stored longer at lower temperatures (4.4° and 10° C), but after 7 days there was considerable effect on the biological characteristics measured. There was also good evidence in a preliminary study that parasitized host larvae could be held for up to 14 days at 13° C before transfer to a 26° C environment. Thus, storage at low temperatures may be possible for up to 4 weeks. In fact, puparia were stored at 15.6° C in 1976 before field release in Louisiana.

PRODUCTION AND COST FACTORS

During the period 1973–76, when tests were being conducted in Louisiana and Florida to determine the feasibility of using *L. diatraeae* in periodic releases for control of the SCB, about 4.4 million *L. diatraeae* were produced during the sugarcane-growing season (March to mid-October) using the methods described (table 6). Large numbers of host insects were required during this same period for colony maintenance and parasite production. Additional parasites and hosts were produced at other times of the year for tests and colony maintenance. The figures in table 6 reflect improvements in rearing techniques and utilization of the GWM as an unnatural host; each succeeding year production was either doubled or tripled. The cost per 1,000 *L. diatraeae* puparia for diet, labor, and rearing

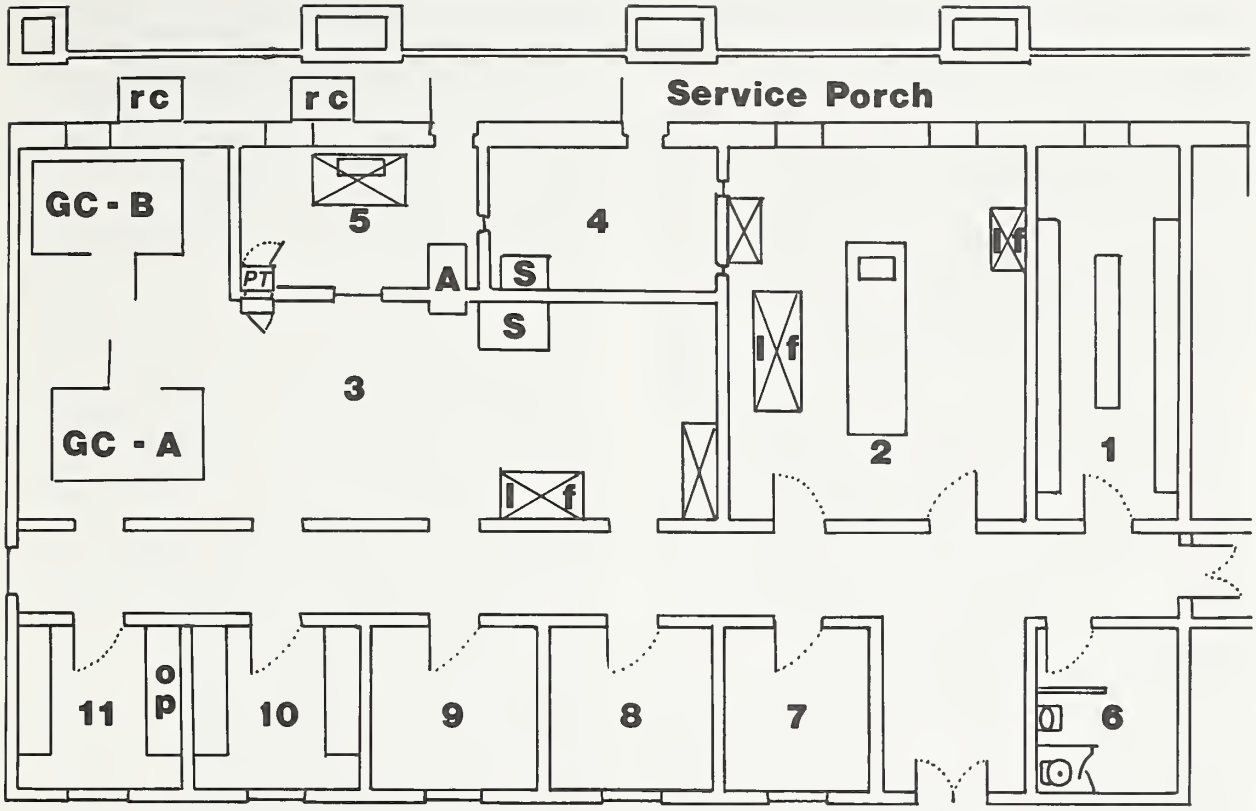

FIGURE 15.—Floor plan of main facility for producing *L. diatraeae*, the sugarcane borer, and the greater wax moth. 1, Storeroom. 2, Diet-dispensing and larval-infesting room. 3, General workroom. 4, Diet sterilization room. 5, Puparia harvest room. 6, Restroom. 7–11, Holding rooms. lf, Laminar-flow hoods. A, Autoclave. GC, Walk-in growth chambers. rc, Refrigeration compressors for walk-in growth chambers. *PT*, Passthrough. OP, Oviposition pallet. S, Sink.

containers was reduced by about 81% when using the GWM rather than the SCB. The savings were achieved primarily in labor and diet costs.

REARING FACILITIES

Areas for producing *L. diatraeae* and some host-insect life stages were described, in part, by Gantt et al. (1978). However, their description did not include rooms for rearing GWM larvae; storing and mixing GWM diet; harvesting parasite puparia, SCB pupae, and GWM larvae; and fly holding. Also, our actual system of production differed significantly from that previously reported.

The insect-production areas used were not originally constructed for insect rearing but were altered from existing space in the U.S. Delta States Agricultural Research Center, Stoneville, Miss. The 232-m^2 main facility (fig. 15) used in this program was located on the first floor and consisted of five 11-m^2 temperature- and RH-controlled insect holding rooms (7–11), restroom, storeroom, hallway, 52-m^2 diet-dispensing and larval-infesting room (2), general workroom (3), harvest room (5), and diet-sterilization room (not used in this program). In addition to other items, the general

Table 6.—Production of the sugarcane borer, greater wax moth, and *L. diatraeae* at Stoneville, Miss., 1973–76[1]

[Thousands]

Year	Sugarcane borer	Greater wax moth	*L. diatraeae*
1973	472	([2])	221
1974	1,117	([2])	677
1975	612	6,713	1,379
1976	684	5,500	2,133
Total	2,885	12,213	4,410

[1]Production during sugarcane growing season (March to mid-October).

[2]Was not reared during 1973–74.

workroom housed two walk-in growth chambers, laminar-flow hood, sink, and fume hood. A pass-through double-door autoclave installed within the wall connected the insect harvest area and the rest of the facility. The diet-dispensing and larval-infesting room contained two laminar-flow hoods, fume hood, and center counter with sink. The harvest room contained a sink and exhaust hood. Three 52-m^2 auxiliary rooms were located elsewhere on the first floor. One of these rooms was equipped with temperature and RH controls, and the other two rooms remained at existing building conditions. Also, three 9-m^2 walk-in environmental rooms on the fourth floor of the building were used.

Though much of the main facility used for rearing was equipped with air filters to supply clean air, only room 2, which was used for mixing, dispensing, and infesting of the soybean flour-wheat germ diet, approximated clean-room conditions. Methods for dispensing the diet were described by Stadelbacher and Brewer (1974). Cups infested with SCB larvae were housed in rooms 3 and 9. Harvesting of parasite puparia and SCB pupae was conducted in one of the three auxiliary rooms. The SCB moths were held in growth chamber A for oviposition, and eggs were harvested underneath the fume hood (room 3) to avoid inhalation of moth scales. Harvested eggs were disinfected and air-dried under the laminar-flow hood (room 3) or in the auxiliary rooms. One of these rooms was used for diet storage and mixing and egg infesting, another room for egg incubating and larval rearing in 3.8-l jars, and the remaining room for GWM larval harvesting for parasitizing and packaging puparia for shipment to Louisiana or Florida. Moth emergence and oviposition cages were housed in room 11.

The larvae of SCB and GWM were infested with *L. diatraeae* maggots in room 3. The cups containing parasitized SCB larvae were maintained in room 7, and trays containing parasitized GWM larvae were held in room 10. Puparia were harvested from the trays in room 5. Those used for the reproductive colony were transferred to cages held in the walk-in environmental chambers on the fourth floor for emerging and holding. Twelve- to fourteen-day-old flies were returned to room 3 for maggot extraction.

The areas described satisfied conditions necessary for production of life stages of three insects. Clean room conditions necessary for handling of the soybean-wheat germ diet were provided in room 2. The five holding rooms (7 to 11) were equipped with humidity and temperature controls that allowed for holding of SCB larvae, parasitized SCB and GWM larvae, and GWM moths. The environmental chambers were necessary in obtaining the high humidity required for SCB moths and *L. diatraeae* flies. The auxiliary room used for rearing GWM larvae was equipped to attain high temperatures (30° C) and maintain a low RH (50%) to promote larval growth and yet prevent mold growth in rearing containers.

DISCUSSION

Mass-rearing procedures were not developed for producing the SCB, which necessitated the use of the GWM for mass production of *L. diatraeae*. A major problem is separating the individual eggs so that they can be placed in separate rearing containers to prevent biting, using a system similar to that reported by Raulston and Lingren (1972). Several attempts were made to separate the eggs using NaOCl or trypsin, but none were successful (unpublished data).

We simplified and reduced the costs of the SCB diet reported by Brewer (1976) by eliminating formaldehyde and potassium hydroxide. Also, choline, chloride, vitamin B suspension, and ascorbic acid were included with other vitamins added to the diet in a premixed dry form. Sorbic acid was added as an antimicrobial agent. Additional cost reductions for diet may be attained by reducing the agar content (Brewer 1976) and replacing the wheat germ with corn oil (Guerra and Bhuiya 1977). We also have evidence that methyl-*p*-hydroxybenzoate may be replaced with 66 mg of benomyl[5] per liter of diet and yet attain better control of fungi that contaminate the diet.

The GWM does lend itself to mass production. Large numbers of eggs can be obtained from moths that require minimum attention, and the larvae can be reared en masse. Our methods, including larval harvesting, can be greatly modified to allow greater automation and production. This should result in considerable cost reduction. Mass production of *L. diatraeae* on the SCB is probably not economically feasible by the procedures we developed, but it is approached on the GWM. However, the ultimate feasibility of these procedures will depend on the number of *L. diatraeae* required per hectare per season for control of the SCB and the availability and cost of alternative methods for controlling this

[5]Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate.

pest. Additional cost reduction for producing GWM larvae may be attained by modifying the diet, and there are many alternatives available. However, the quality of the parasite reared on GWM larvae that are fed the various diets should be evaluated, since parasite vigor can be affected by its host source and the food fed to the host.

Facility requirements are dictated by the need for sanitation and the environmental requirements of the insects and insect production workers. Requirements vary according to each individual program. We are presently altering the facility described for mass-producing *Heliothis* species. Consistent temperatures within rearing areas are important so that production can be programed to provide specific numbers of insects at specific times. Requirements for the different species differ, and what is optimum for one life stage may not be optimum for another, even within species. For example, an area had to be provided for rearing the GWM at or above 30° C, but a lower temperature (28° C) was needed for rearing the SCB and maggot stage of *L. diatraeae*. Also, the adult stage of the SCB and *L. diatraeae* required a lower temperature for high survival, but development and egg production also had to be considered; thus, 24° to 26° C was selected for holding these two stages. High humidity was required for high survival of *L. diatraeae* and SCB adults, but a lower humidity was desirable in host larval-rearing areas to reduce microbial contamination. Clean-room conditions were absolutely necessary only where the soybean flour-wheat germ diet was poured and infested with SCB larvae, but other areas were kept clean and orderly. The working areas were kept at about 25° C for personnel, and movement was limited in the diet-pouring areas because of the need for cleanliness, in the GWM larval-rearing areas because of the high temperatures, and in the moth-holding areas because moth scales constituted a health hazard.

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